

**INTERACTION-ACTIVATED, CIRCULARLY PERMUTATED PROTEINS****ABSTRACT OF THE DISCLOSURE**

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Interaction-activated circularly permuted proteins are disclosed that depend for their functional reassembly into the parent protein on the interaction of heterologous polypeptides or other molecules which have been genetically or chemically conjugated to the break-point termini of engineered enzymes. In addition, methods are provided for identifying circularly permuted marker proteins that will optimally reassemble into a functional parent protein, and which are dependent on the association of heterologous interactor domains. The invention is exemplified by circular permutations of a Class A  $\beta$ -lactamase (TEM-1 of *E. coli*). Circularly permuted marker proteins that comprise molecular interaction-dependent enzymes particularly find use in (1) cell-based sensors for activation or inhibition of metabolic or signal transduction pathways for high-efficiency, (2) high-throughput screening for agonists/antagonists of the target pathway and in high-throughput mapping of pair-wise protein-protein interactions within and between the proteomes of cells, tissues, and pathogenic organisms, and in (3) cell-based screens for high-throughput selection of inhibitors of any protein-protein interaction.

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